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# Hormesis in the brown citrus aphid, *Toxoptera citricida* (Kirkaldy) (Hemiptera: Aphididae) exposed to sublethal doses of imidacloprid

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## Abstract

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is a major pest that transmits phloem-limited, gram negative bacteria including 'Candidatus' *Liberibacter asiaticus* (Clas), causing huanglongbing. Management of this pest relies primarily on insecticides, such as the neonicotinoid imidacloprid, that may affect secondary pests including the brown citrus aphid, *Toxoptera citricida* (Kirkaldy) (Hemiptera: Aphididae). Here, we report on direct toxicity and sublethal dose effects of imidacloprid on *T. citricida* nymphs and adults following direct contact and ingestion. We also examined transgenerational dose-response and hormetic effects following exposure of *T. citricida* to a sublethal concentration of imidacloprid. Toxicity of imidacloprid was similar for nymphs (0.6 ng per  $\mu\text{L}$ ) and adults (1 ng per  $\mu\text{L}$ ) at 72 h. Fecundity and finite rate of increase were greater for populations exposed to systemic and foliar treatments at a sublethal concentration (0.1 ng per  $\mu\text{L}$ ) compared with untreated controls. Development times of first instar nymphs in the  $F_1$  generation and third instar nymphs in the  $F_2$  generation were significantly greater for individuals treated with the sublethal dose than an untreated control. Survival of second instar *T. citricida* on plants treated with a sublethal concentration also was significantly greater than controls. There also was a significant increase in fecundity of  $F_1$  and  $F_2$  individuals after sublethal treatment compared with controls. Our results indicated that a sublethal concentration of imidacloprid increased the reproductive performance and induced possible physiologically stimulative (hormetic) effects in *T. citricida*. Hormesis in secondary pests should be considered when developing a management program for pathogen vectors such as *D. citri*.

Key Words: secondary pests; stimulated reproduction; stress conditioning; toxicity; huanglongbing

## Resumen

El psílido asiático de los cítricos, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), es una plaga importante que transmite bacterias gramnegativas limitadas por el floema, incluyendo *Liberibacter asiaticus* (Clas), *Candidatus*, causando la enfermedad huanglongbing. El manejo de esta plaga se basa principalmente en insecticidas, como el imidacloprid neonicotinoide, que puede afectar a las plagas secundarias, incluido el pulgón marrón de los cítricos, *Toxoptera citricida* (Kirkaldy) (Hemiptera: Aphididae). Aquí, informamos sobre la toxicidad directa y los efectos de la dosis subletal de imidacloprid en ninfas de *T. citricida* y adultos después del contacto directo y la ingestión. También examinamos la respuesta a la dosis transgeneracional y los efectos horméticos después de la exposición de *T. citricida* a una concentración subletal de imidacloprid. La toxicidad de imidacloprid fue similar para las ninfas (0.6 ng por  $\mu\text{L}$ ) y adultos (1 ng por  $\mu\text{L}$ ) a las 72 h. La fecundidad y la tasa finita de aumento fueron mayores para las poblaciones expuestas a tratamientos sistémicos y foliares a una concentración subletal (0.1 ng por  $\mu\text{L}$ ) en comparación con los controles no tratados. Los tiempos de desarrollo de las ninfas de primer estadio en la generación  $F_1$  y las ninfas del tercer estadio en la generación  $F_2$  fueron significativamente mayores para los individuos tratados con la dosis subletal que un control no tratado. La sobrevivencia del segundo estadio de *T. citricida* en plantas tratadas con una concentración subletal también fue significativamente mayor que los controles. También hubo un aumento significativo en la fecundidad de los individuos  $F_1$  y  $F_2$  después del tratamiento subletal en comparación con los controles. Nuestros resultados indicaron que una concentración subletal de imidacloprid aumentó el rendimiento reproductivo e indujo posibles efectos fisiológicamente estimulantes (horméticos) en *T. citricida*. Se debe considerar la hormesis en plagas secundarias al desarrollar un programa de manejo para vectores de patógenos como *D. citri*.

Palabras Claves: plagas secundarias; reproducción estimulada; acondicionamiento de estrés; toxicidad; huanglongbing

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The brown citrus aphid, *Toxoptera citricida* (Kirkaldy) (Hemiptera: Aphididae), is considered one of the most important vectors of citrus tristeza virus in citrus (Michaud 1998). This species transmits citrus tristeza virus with high efficiency (Roistacher & Bar-Joseph 1987; Bar-Joseph et al. 1989). Citrus tristeza virus is a phloem-limited closterovirus and some strains can cause devastating losses in citrus, particularly in regions using a sour orange rootstock (Tsai & Wang 1999). *Toxoptera citricida* is found throughout Europe, Asia, Pacific Islands, Sub-Sahara Africa, and South America (Carver 1978). It spread through Central America and the Caribbean islands during the late 1980s (Yokomi et al. 1994), and was first detected in southern Florida in 1996 (Halbert & Brown 1996) where it rapidly became distributed throughout the citrus producing regions of the state (Liu & Tsai 2002). In Florida groves, the brown citrus aphid is restricted to host plants in the Rutaceae family where it is responsible for the transmission of citrus tristeza virus to citrus trees (Brlansky et al. 2003; Halbert et al. 2004). Citrus tristeza virus causes stem-pitting, quick decline, and reduces productivity of infected trees. In the 1960s, sour orange rootstock was planted widely in Florida citrus groves allowing citrus tristeza virus to become widespread and remain endemic. Since then, sour orange rootstock has been identified as the most susceptible rootstock to citrus tristeza virus and has been replaced with more tolerant alternatives (e.g., Carrizo and Swingle trifoliate, as well as others). Although the incidence of citrus decline has decreased, the viral population of citrus tristeza virus strain T36 is still endemic in Florida groves (Harper & Cowell 2016). This could lead to a resurgence in citrus decline from citrus tristeza virus, because this disease agent has not been eradicated (Shivankar et al. 2015).

Neonicotinoid insecticides applied as foliar sprays and soil drenches have been used widely since the early 1990s for management of a broad spectrum of sucking insect pests (Nauen et al. 1998; Jeschke et al. 2011). Imidacloprid, for example, has been an important insecticide for control of Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae). This psyllid is the vector of *Candidatus liberibacter asiaticus*, the putative agent causing huanglongbing in Florida (Grafton-Cardwell et al. 2013). Application of imidacloprid also will control other secondary citrus pests such as *T. citricida*. Resistance management for *D. citri* has emerged as a recent challenge (Grafton-Cardwell et al. 2013; Langdon & Rogers 2017; Chen et al. 2018); however, the possibility of secondary pest outbreaks associated with insecticide overuse for management of *D. citri* has received relatively little attention.

Secondary pests are exposed to lethal and sublethal concentrations of insecticides (Desneux et al. 2005). Such exposure may result in unintended consequences on pests, such as hormesis, which is a biphasic dose response relationship where lower concentrations are stimulatory causing pest outbreaks and where higher concentrations are lethal (Guedes et al. 2016). Hormesis is of interest in insect pest management because breakdown of insecticides in agricultural fields will expose pests to low or sublethal concentrations of these products. Hormesis has been found within all groups of organisms, and it is induced by physical and chemical stress factors including those caused by many insecticides and phytotoxins (Chen & Nakasuji 2004; Calabrese 2005; Chen et al. 2017). Hormesis resulting in pest resurgence could not only result in increased crop and commodity damage, but may also lead to additional pesticide treatments potentially exacerbating non-target impacts, insecticide resistance, and environmental contamination. Increasing survival, fecundity, and reproduction following exposure to sublethal concentrations of insecticides have been reported in several pest and beneficial insects (Luckey 1968; Morse & Zareh 1991; Cutler et al. 2005).

Various lethal and sublethal effects of imidacloprid have been reported recently in several insects and mites such as a mirid bug, *Apolygus lucorum* (Meyer-Dür) (Hemiptera: Miridae) (Tan et al. 2012; Pan et al. 2014); tobacco whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (He et al. 2013); cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) (Shi et al. 2011); green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Ayyanath et al. 2013); English grain aphid, *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae) (Miao et al. 2013); and a predatory mite, *Amblyseius victoriensis* (Womersley) (Acarina: Phytoseiidae) (James 1997). However, potential sublethal effects of imidacloprid on *T. citricida* are unknown. In Florida, we hypothesized that long-term use of imidacloprid to manage *D. citri* may have exposed *T. citricida* to sublethal concentrations, thus increasing population growth rates of *T. citricida* in unpredictable ways depending on factors such as hormesis and increased citrus tristeza virus infection. The goal of this investigation was to quantify the sublethal effects of imidacloprid on *T. citricida* to facilitate inclusion of this phytopathogen vector into current citrus integrated pest management programs.

## Materials and Methods

### INSECTS AND PLANTS

*Toxoptera citricida* used in these experiments originated from a population maintained on Mexican orange, *Choisya ternata* H.B.K. (Rutaceae), plants (Forest Farm, Williams, Oregon, USA) in a greenhouse facility at the University of Florida Citrus Research and Development Center, Lake Alfred, Florida, USA. This population has been reared in the laboratory without exposure to insecticides since Jun 2017. Aphids were reared on *Choisya ternata* (Rutaceae) and 'Swingle citrumelo' (*Citrus paradisi* Macf. × *Poncirus trifoliata* [L.] Raf.; both Rutaceae) plants maintained in a rearing room at 25 ± 2 °C, 16:8 h (L:D) photoperiod, and 65 ± 5% RH.

### INSECTICIDE ACUTE TOXICITY ASSESSMENT

Imidacloprid formulated as Admire pro 4.6F was obtained from Bayer Crop Science (Leverkusen, Germany). Admire pro 4.6 was suspended in deionized water to make a 1,000 mg AI per L stock solution that was diluted to make experimental solutions of 5 to 8 concentrations for bioassays and toxicity testing. Each insecticide concentration was diluted with deionized water; the control treatment was deionized water only. Leaves were collected from *Citrus sinensis* L. Osbeck (Rutaceae) cv 'Valencia' orange trees that had not been treated with insecticide for at least 3 yr, and 35 mm diam leaf discs were cut from these leaves. A 1.5% agar solution was poured into 35 mm Petri dishes (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to form a bed after solidification to prevent leaf senescence. Leaf discs were dipped in test solutions for 15 to 30 s then placed into the prepared Petri dishes (McKenzie 2004). Each concentration of imidacloprid was replicated 4 to 5 times and the entire experiment was replicated twice. Approximately 10 nymphs or adult *T. citricida* were added to each Petri dish to determine mortality. In total, 600 to 700 aphid nymphs and adult individuals were tested. Mortality counts for nymphs and adults were recorded at 24, 48, and 72 h after transfer into a growth chamber at the environmental conditions described above for insect rearing. Individuals that did not move their legs after being touched with a fine brush were considered dead.

## POPULATION GROWTH OF *TOXOPTERA CITRICIDA* ON PLANTS WITH SYSTEMIC OR FOLIAR TREATMENT

To test the effects of sublethal concentrations of imidacloprid on *T. citricida* reproduction, systemic treatment of water or imidacloprid solution (50 mL) was watered onto soil in pots (110 mm diam; 130 mm height; 181.01 ± 7.46 g soil weight) containing individual 15 cm high 'Swingle citrumelo' (*C. paradisi* Macf. × *P. trifoliata* L. Raf.) plants. One d after treatments were applied, 5 adult aphids were placed onto each plant. Each branch containing an adult aphid was enveloped with a fine mesh bag tied at the stem. *Toxoptera citricida* were allowed to develop and reproduce on plants under rearing conditions for 7, 14, and 21 d, and were counted daily. This was a sufficient duration for the development of 3 generations.

For foliar applications, 5 *T. citricida* adults were placed on a potted plant and sprayed with 5 mL of water or insecticide solution. After each treatment, individuals were transferred into a cage (60 × 30 × 30 cm) containing 1 untreated 'Swingle' plant. Aphids were maintained for 3 generations. There were 4 replications per treatment. After 7, 14, and 21 d, the total number of aphids on each plant was counted. The instantaneous rate of increase ( $r_i$ ) was calculated for each aphid, using the following formula:

$$r_i = \ln(N_t/N_0)/T$$

where  $N_t$  was the final number of aphids on a plant,  $N_0$  was the initial number of aphids introduced to the plant, and  $T$  was the exposure time (Tsai & Wang 1999; Chen et al. 2010; Chen & Stark 2010; Ayyanath et al. 2013).

## CONCENTRATION-RESPONSE MODELING

In addition to analysis of variance methods, we used a modified 4-parameter logistic model developed to test for hormesis and to assess the concentration at which maximal hormetic response occurred (Schabenberger et al. 1999; Ayyanath et al. 2013; Nweke & Ogbonna 2017). This was performed on 21 d mean fecundity data from the leaf dip experiment, as well as soil and foliar application experiments using the following equation:

$$E[Y]x = \delta + \frac{\alpha - \delta}{1 + \exp[\beta \ln(x) + x]}$$

where  $\delta$  is the lower limit (0) of the dose-response curve;  $\alpha$  represents the steepness of the curve after the maximal hormetic effect;  $x$  provides a lower bound on the sublethal dose level; and  $\beta$  is the point of inflection of the curve. Parameter  $E[Y]$  cannot be considered a direct representation of the extent of hormesis, but  $\beta > 0$  suggests presence of hormesis (Schabenberger et al. 1999; Ayyanath et al. 2013; Nweke & Ogbonna 2017).

## SUBLETHAL EFFECTS OF IMIDACLOPRID ON DEVELOPMENTAL TIME OF *TOXOPTERA CITRICIDA*

In order to demonstrate concentration-mortality effects of individuals, approximately 500 adult aphids were placed onto 4 Swingle plants for 24 h and then removed to allow nymphs to develop into adults. From these emerging nymphs, approximately 300  $F_0$  generation adults were subjected to a sublethal concentration (0.1 ng per  $\mu$ L) of imidacloprid on the leaf disc or a water control. Treatments were applied to freshly cut untreated citrus leaves placed on 1.5% agar within Petri dishes where emerging  $F_0$  generation adults were allowed to produce new  $F_1$  nymphs over 24 h. After 24 h,  $F_0$  adults

and  $F_1$  nymphs were removed until only 1 nymph remained per Petri dish. Fresh Swingle citrumelo leaves were supplied to each Petri dish every d.  $F_1$  nymphs were reared to adults and  $F_2$  generation aphids of the same age were tested using the above procedure in order to quantify sublethal effects of imidacloprid on developmental time during 2 consecutive generations of treatment-exposed aphids. Petri dishes with aphids were placed in a growth chamber at 25 ± 2 °C, 60 ± 10% RH, and with a 16:8 h (L:D) photoperiod. This experiment was replicated 30 to 50 times. Development times were recorded daily as earlier mentioned.

## SUBLETHAL EFFECTS OF IMIDACLOPRID ON FECUNDITY OF INDIVIDUAL $F_1$ AND $F_2$ *TOXOPTERA CITRICIDA*

This experiment quantified fecundity using a different cohort of insects but generally followed the study design described earlier wherein the offspring produced by the  $F_0$  adults were collected and used as the  $F_1$  generation. Aphids were retained on citrus leaf discs without insecticide and allowed to feed on newly prepared Swingle citrumelo leaves daily. The  $F_1$  nymphs were reared into  $F_2$  adults of the same known age. The  $F_2$  generation aphids were tested using the above procedure in order to quantify sublethal effects of imidacloprid on fecundity of treatment-exposed aphids. This procedure was repeated 30 times for leaves treated with 0.1 ng per  $\mu$ L of imidacloprid as described earlier or the water control. Replicates were individual aphids. Newly emerged nymphs were counted and removed daily (only adult aphids remained) until the death of the adult. Adult fecundity was quantified at 1, 2, and 3 d after exposure to treatments.

## EFFECT OF SUBLETHAL IMIDACLOPRID CONCENTRATION ON SURVIVAL OF VARIOUS INSTARS

This experiment was conducted using a different cohort of individuals from those described earlier but also followed the above protocol initially by placing approximately 500  $F_0$  adults onto Swingle citrumelo plants. Three hundred  $F_1$  nymphs were subsequently removed after 24 h and placed onto 1 to 2 yr old Swingle seedlings with new flush. Adult aphids were treated with either a sublethal dose (0.1 ng per  $\mu$ L) of imidacloprid or a water control using the leaf dip Petri dish assay method described earlier. There were 20 replicates that consisted of 1 adult aphid per leaf within Petri dishes for treatment and control. After 24 h, adults were removed and newly emerging nymphs were counted daily by instar. Adult mortality was recorded until emergence of the  $F_2$  generation was complete. Survival of various instars from first instar nymphs to adults was determined.

## STATISTICAL ANALYSIS

Mortality data were subjected to probit analysis (Finney 1971) and processed using PROC probit SAS 9.4 (SAS Institute 2002-2012). Abbott's formula (Abbott 1925) was used to adjust for mortality in controls when it occurred. Statistical differences between  $LC_{50}$  values were determined using the presence or absence of overlap in the 95% fiducial limits. Differences between treatments for development time, survival rate, and nymphs produced per female adult were compared using Student's  $t$ -tests (SAS Institute 2002-2012). Fecundity and instantaneous rate of increase were analyzed using a 2-way, mixed model analysis of variance. The first order interaction was removed from the analytical model if it was not significant (Sokal & Rohlf 1995). If the first order interaction was significant, data were subjected to the Bonferroni test in each treatment and control (Sokal & Rohlf 1995). Differences in all analyses were considered significant at  $P = 0.05$ .

## Results

### ACUTE TOXICITY OF IMIDACLOPRID IN LEAF DIP BIOASSAY ON *TOXOPTERA CITRICIDA*

Levels of toxicity exhibited by imidacloprid to nymph and adult *T. citricida* are given in Table 1. The  $LC_{50}$  of imidacloprid gradually, but significantly ( $P = 0.031$ ), decreased from 11.73 to 1 ng per  $\mu\text{L}$  between 24 and 72 h after exposure, and 10.61 to 0.60 ng per  $\mu\text{L}$  between 24 and 72 h after exposure for adults and nymphs, respectively. Similarly, the  $LC_{95}$  of this insecticide gradually decreased from 6,802 to 789.31 ng per  $\mu\text{L}$  between 24 to 72 h after exposure, and 15,305 to 408.12 ng per  $\mu\text{L}$  between 24 and 72 h after exposure for adults and nymphs, respectively.

### EFFECT OF SYSTEMIC AND FOLIAR EXPOSURE TREATMENTS

There were no significant differences in fecundity due to the different concentrations of imidacloprid applied to soil at 7 ( $F_{5,18,23} = 0.06$ ;  $P = 0.997$ ), 14 ( $F_{5,18,23} = 0.15$ ;  $P = 0.978$ ), and 21 ( $F_{5,18,23} = 0.43$ ;  $P = 0.82$ ) d after exposure (Table 2). There were no significant differences in fecundity due to the different concentrations of imidacloprid applied by foliar spray at 7 ( $F_{5,18,23} = 2.61$ ;  $P = 0.06$ ), 14 ( $F_{5,18,23} = 1.71$ ;  $P = 0.18$ ), and 21 ( $F_{5,18,23} = 2.21$ ;  $P = 0.098$ ) d after exposure. The finite rate of population increase was not affected by the concentration of imidacloprid applied to soil at 7 ( $F_{5,18,23} = 0.29$ ;  $P = 0.92$ ), 14 ( $F_{5,18,23} = 0.62$ ;  $P = 0.68$ ),

or 21 ( $F_{5,18,23} = 0.11$ ;  $P = 0.989$ ) d after exposure (Table 3). Concentrations of imidacloprid applied as foliar treatment had no significant effect on total fecundity at 7 ( $F_{5,18,23} = 2.44$ ;  $P = 0.074$ ), 14 ( $F_{5,18,23} = 1.68$ ;  $P = 0.19$ ), and 21 ( $F_{5,18,23} = 1.85$ ;  $P = 0.15$ ) d after exposure as compared with controls. Although fecundity measures did not, in most cases, differ statistically across concentrations, we found a highly reproducible trend for a hormetic peak with decreasing fecundity values toward the lowest and highest concentrations tested (Table 3).

### SUBLETHAL EFFECTS OF IMIDACLOPRID ON *TOXOPTERA CITRICIDA*

Developmental time of first instar nymphs treated with the sublethal concentration of imidacloprid was significantly longer ( $P = 0.005$ ) than in controls for the  $F_1$  generation; however, this was not observed for second, third, or fourth instars (Table 4). There was a consistent pattern wherein the treatment caused a delay in developmental time compared with the control (Table 4). For the  $F_2$  generation, developmental time was significantly longer for third instars than in the control ( $P = 0.035$ ); however, no statistical differences were observed with any of the other instars.

Survival rates of individual  $F_1$  *T. citricida* exposed to the sublethal concentration of imidacloprid are given in Table 5. There was a significant increase in survivorship of second instar nymphs compared with the control (Table 5). There were no statistical differences observed for the other immature stages; however, there was a general trend of greater survival in exposed individuals compared with control aphids (Table 5).

**Table 1.** Mean toxicity levels (and confidence intervals) of imidacloprid for control of *Toxoptera citricida* as measured by leaf dip bioassay.

Time (h) after exposure	Stage	Slope	$LC_{50}$ (ng per $\mu\text{L}$ ) (95% FL)*	$LC_{95}$ (ng per $\mu\text{L}$ ) (95% FL)**
24	Adult	0.60 $\pm$ 0.13	11.73 a (1.61 – 178.88)	6802.00 (34355.00 – 4.94 $\times$ 10 <sup>7</sup> )
	Nymph	0.52 $\pm$ 0.10	10.61 b (1.57 – 160.07)	15305.00 (602.66 – 1.04 $\times$ 10 <sup>8</sup> )
48	Adult	0.40 $\pm$ 0.08	2.02 b (0.53 – 30.79)	1044.00 (95.29 – 1.04 $\times$ 10 <sup>6</sup> )
	Nymph	0.56 $\pm$ 0.09	1.18 b (0.34 – 6.25)	1049.00 (95.62 – 1.93 $\times$ 10 <sup>5</sup> )
72	Adult	0.61 $\pm$ 0.08	1.00 b (0.25 – 3.77)	789.31 (90.25 – 52079.00)
	Nymph	0.62 $\pm$ 0.11	0.60 c (0.10 – 2.22)	408.12 (45.55 – 36425.00)

\*Means within a column followed by the same letter are not statistically different ( $P = 0.05$ ).

\*\*Statistical analyses not conducted at this lethal concentration due to high variability of 95% fiducial limits.

**Table 2.** Mean fecundity  $\pm$  SE of *Toxoptera citricida* per plant 7, 14, and 21 d following exposure to citrus plants treated with various concentrations (0.001–10 ng per  $\mu\text{L}$ ) of imidacloprid and water control with soil or foliar application.

Concentration ( $\mu\text{g}$ per $\mu\text{L}$ )	Founding number	Mean number of eggs*		
		7 d	14 d	21 d
Soil application				
0	25	6.75 $\pm$ 1.88 a	14.75 $\pm$ 7.54 a	31.25 $\pm$ 19.41 a
0.001	25	7.50 $\pm$ 2.87 a	11.25 $\pm$ 4.99 a	23.50 $\pm$ 16.74 a
0.01	25	5.75 $\pm$ 3.75 a	17.25 $\pm$ 8.76 a	33.50 $\pm$ 19.50 a
0.1	25	7.50 $\pm$ 5.33 a	23.50 $\pm$ 17.86 a	95.75 $\pm$ 49.28 a
1	25	8.75 $\pm$ 5.54 a	21.50 $\pm$ 15.96 a	22.75 $\pm$ 17.20 a
10	25	5.50 $\pm$ 2.63 a	16.00 $\pm$ 9.27 a	10.00 $\pm$ 6.00 a
Foliar application				
0	25	7.25 $\pm$ 2.98 a	7.00 $\pm$ 2.04 a	16.75 $\pm$ 11.77 a
0.001	25	35.00 $\pm$ 18.65 a	18.25 $\pm$ 10.63 a	27.50 $\pm$ 15.88 a
0.01	25	33.75 $\pm$ 13.71 a	33.25 $\pm$ 17.59 a	61.50 $\pm$ 38.34 a
0.1	25	42.25 $\pm$ 7.63 a	50.75 $\pm$ 9.44 a	148.25 $\pm$ 36.14 a
1	25	12.50 $\pm$ 6.43 a	30.00 $\pm$ 14.51 a	91.50 $\pm$ 41.17 a
10	25	2.75 $\pm$ 1.75 a	13.50 $\pm$ 12.50 a	27.75 $\pm$ 27.75 a

\*Means within a column followed by the same letter are not statistically different ( $P = 0.05$ ).

**Table 3.** Instantaneous mean  $\pm$  SE rate of increase ( $r_t$ ) of *Toxoptera citricida* per plant 7, 14, and 21 d following exposure to citrus plants treated with various concentrations (0.001–10 ng per  $\mu$ L) of imidacloprid and water control by soil or foliar application.

Concentration* ( $\mu$ g per $\mu$ L)	Founding number	Instantaneous rate of increase $\pm$ SE*		
		7 d	14 d	21 d
Soil application				
0	25	0.02 $\pm$ 0.05 a	0.03 $\pm$ 0.06 a	0.02 $\pm$ 0.06 a
0.001	25	0.03 $\pm$ 0.05 a	-0.03 $\pm$ 0.04 a	0.01 $\pm$ 0.05 a
0.01	25	-0.05 $\pm$ 0.07 a	-0.04 $\pm$ 0.05 a	0.02 $\pm$ 0.06 a
0.1	25	0.06 $\pm$ 0.11 a	0.02 $\pm$ 0.07 a	0.12 $\pm$ 0.03 a
1	25	-0.01 $\pm$ 0.08 a	-0.04 $\pm$ 0.06 a	0.03 $\pm$ 0.04 a
10	25	-0.07 $\pm$ 0.10 a	0.01 $\pm$ 0.07 a	-0.01 $\pm$ 0.05 a
Foliar application				
0	25	0.02 $\pm$ 0.05 ab	0.03 $\pm$ 0.02 a	0.03 $\pm$ 0.03 a
0.001	25	0.15 $\pm$ 0.14 ab	0.01 $\pm$ 0.07 a	0.02 $\pm$ 0.06 a
0.01	25	0.17 $\pm$ 0.14 ab	0.08 $\pm$ 0.07 a	0.07 $\pm$ 0.05 a
0.1	25	0.30 $\pm$ 0.03 a	0.16 $\pm$ 0.02 a	0.16 $\pm$ 0.02 a
1	25	0.05 $\pm$ 0.10 ab	0.07 $\pm$ 0.07 a	0.10 $\pm$ 0.06 a
10	25	-0.16 $\pm$ 0.07 b	-0.05 $\pm$ 0.07 a	-0.02 $\pm$ 0.06 a

\*Means within a column followed by the same letter are not statistically different for soil and foliar applications ( $P = 0.05$ ).

**Table 4.** Mean developmental  $\pm$  SE time of various life stages for  $F_1$  generation *Toxoptera citricida* when parents ( $F_0$ ) were exposed to a sublethal concentration (0.1 ng per  $\mu$ L) of imidacloprid compared with water controls.

Generation	Stage	Control*		Imidacloprid		$P$	$t$ -value	df
		$n$	(d)	$n$	(d)			
$F_1$	1st instar	30	1.13 $\pm$ 0.06 a	30	1.43 $\pm$ 0.22 b	0.005	747.00	59
	2nd instar	15	1.68 $\pm$ 0.25 a	16	2.00 $\pm$ 0.26 a	0.182	-0.92	29
	3rd instar	13	2.08 $\pm$ 0.27 a	14	2.14 $\pm$ 0.21 a	0.784	187.50	26
	4th instar	7	2.14 $\pm$ 0.26 a	7	2.29 $\pm$ 0.49 a	0.805	50.00	14
$F_2$	1st instar	50	1.76 $\pm$ 0.06 a	50	1.82 $\pm$ 0.08 a	0.467	2,450.00	99
	2nd instar	41	1.12 $\pm$ 0.08 a	38	1.24 $\pm$ 0.08 a	0.090	1,624.00	78
	3rd instar	20	2.10 $\pm$ 0.19 a	15	2.73 $\pm$ 0.27 b	0.035	331.00	34
	4th instar	18	1.94 $\pm$ 0.21 a	13	2.00 $\pm$ 0.27 a	0.844	213.00	32

\*Means within a row followed by the same letter are not statistically different ( $P = 0.05$ ).

**Table 5.** Mean survivorship (%)  $\pm$  SE of immature stages of *Toxoptera citricida* following treatment of the parents with a sublethal concentration (0.1 ng per  $\mu$ L) of imidacloprid compared with water control.

Treatment	Percent survivorship			
	1st instar*	2nd instar	3rd instar	4th instar
Control	75.29 $\pm$ 10.08 a	80.40 $\pm$ 5.57 a	88.52 $\pm$ 3.22 a	84.72 $\pm$ 0.11 a
Imidacloprid	77.26 $\pm$ 6.58 a	90.61 $\pm$ 4.20 b	91.94 $\pm$ 4.42 a	89.85 $\pm$ 2.12 a
$t$	395.00	157.00	172.00	62.50
df	41	31	29	25
$P$	0.382	0.048	0.495	0.369

\*Means within a column followed by the same letter are not statistically different ( $P = 0.05$ ).

Maximum fecundity ( $\beta > 0$ ;  $P < 0.05$ ) of *T. citricida* adults occurred at a sublethal exposure concentration of 0.1 ng per  $\mu$ L of imidacloprid in 21 d old adults for soil and foliar treatments (Table 6). Fecundity of treated  $F_1$  and  $F_2$  individuals exposed to a sublethal dose of imidacloprid was significantly higher than in the control at all 3 time points after exposure (Fig. 1A, B).

## Discussion

Hormesis is a biphasic response to a given stressor over a range of concentrations where a stimulatory or potentially beneficial effect is

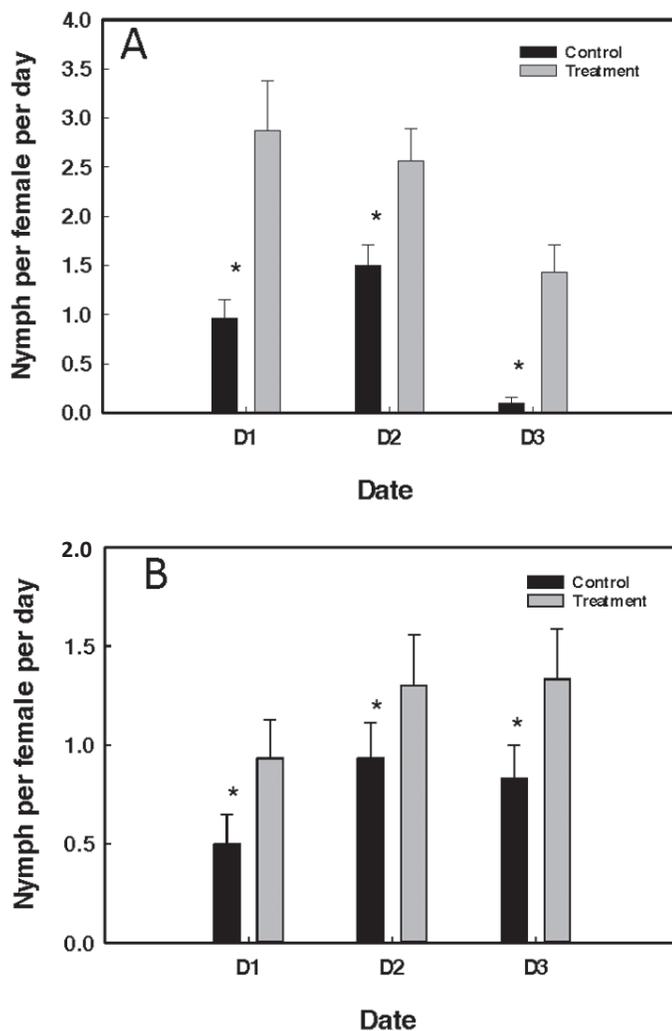
associated with exposure to low concentrations, while an inhibitory or negative effect is observed at high levels of exposure (Calabrese & Baldwin 2002; Costantini et al. 2010; Suhett et al. 2011; Guedes et al. 2016). We observed an increase in fecundity when *T. citricida* adults were exposed to plants treated with a sublethal concentration of imidacloprid by soil drenching or as a foliar spray. The number of nymphs produced per female indicated that the sublethal concentration increased fecundity of  $F_1$  and  $F_2$  *T. citricida* compared with baseline levels (water control). This increase could be induced by the up-regulation of detoxifying enzymes such as esterases or cytochrome P450s (Mukherjee et al. 1993; Chen et al. 2017) or other developmental enzymes and proteins (Smirnoff 1983). Stimulatory effects

**Table 6.** Regression parameters of model-fitting hormetic response (fecundity on d 21) in *Toxoptera citricida* exposed to various concentrations (0.001–10 ng per  $\mu$ L) of imidacloprid and water control with soil or foliar application.

Application	Parameter <sup>a</sup>	Estimate	F-value	P
Soil application	$\alpha$	40.89	5.26	0.047
	$\beta$	4.64		
	df	3, 17		
Foliar application	$\alpha$	92.28	3.29	0.046
	$\beta$	4.75		
	df	3, 17		

<sup>a</sup> $\alpha$  = Steepness of the curve after the maximal hormetic effect;  $\beta$  = rate of stimulation (slope); df = degrees of freedom.

of low concentrations of pesticides (imidacloprid in particular) have been reported in various arthropod groups including *A. victoriensis* (James 1997), *Tetranychus urticae* Koch (Prostigmata: Tetranychidae) (James & Price 2002), *Tryporyza incertulas* (Walker) (Lepidoptera:



**Fig. 1.** Mean number of F<sub>1</sub> (A) and F<sub>2</sub> (B) nymphs per female *Toxoptera citricida* treated with a sublethal concentration (0.1 ng per  $\mu$ L) of imidacloprid compared with water control at 3 intervals after emergence. \*Indicates significant difference between control treatment ( $P < 0.05$ , Student's  $t$ -test).

Crambidae) (Wang et al. 2005), *M. persicae* (Kerns & Gaylor 1992; Cutler et al. 2009), *A. lucorum* (Tan et al. 2012), and cotton aphids, *A. gossypii* (Kerns & Gaylor 1992).

Insecticides will degrade naturally following field application (Desneux et al. 2005; Biondi et al. 2012) which results in exposure of surviving organisms to sublethal concentrations. This sublethal exposure and resulting effects on the agroecosystem requires attention when developing effective integrated pest management programs (Desneux et al. 2005; Biondi et al. 2012). Exposure of secondary pests to sublethal concentrations may affect their fecundity, survival rate, and development time, as well as their susceptibility to natural enemies (Desneux et al. 2007; Guedes et al. 2016). Our data obtained from the dose-response model is the basic foundation for assuming hormesis because the lower concentration of imidacloprid stimulated reproduction whereas the higher concentration caused the opposite effect in *T. citricida*. Hormesis often is challenging to clearly demonstrate (Calabrese 2005). Although the sample size in our investigation was relatively high (30–50 replicate individuals per treatment), it is possible that increasing this sample size further could have more clearly resolved hormesis statistically.

Hormesis has been recognized as a potential link to pest outbreaks and, therefore, it deserves careful attention if pest resurgences are observed, and when developing resistance management protocols (Guedes et al. 2010, 2016). Although resurgence due to insecticide overuse may be less common among aphids (Kerns & Stewart 2000) than other pests, such as citrus thrips, *Scitothrips citri* (Moulton) (Thysanoptera: Thripidae) (Morse & Zareh 1991), yellow stem borer, *Tryporyza incertulas* (Walker) (Wang et al. 2005), and diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Fujiwara et al. 2002), it has been reported in aphids following sublethal insecticide exposure (Cutler et al. 2009). Therefore, additional work on direct toxicity of neonicotinoids, as well as the molecular and physiological mechanisms underlying hormesis in *T. citricida* is warranted for development of appropriate integrated pest management practices that include this pest.

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